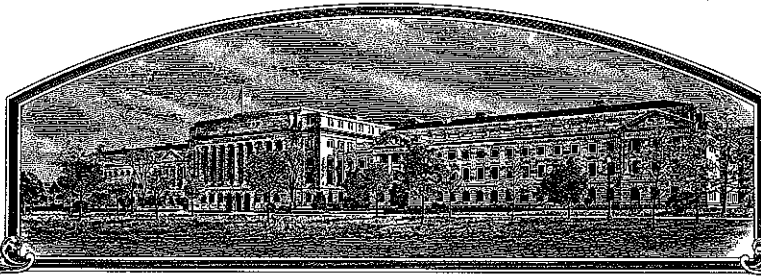


No.

200200013



# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Central Valley Seeds, Inc.

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER-PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSES, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

LETTUCE

'Big Star'

In Testimony Whereof, I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affixed at the City of Washington, D.C. this twelfth day of December, in the year two thousand and five.

Attest:



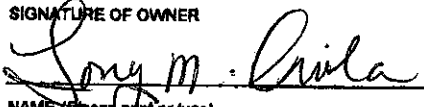

*Alm J.*  
Commissioner  
Plant Variety Protection Office  
Agricultural Marketing Service

*Mill Johnson*  
Secretary of Agriculture

U.S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL MARKETING SERVICE  
SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICEAPPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE  
(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF OWNER <b>CENTRAL VALLEY SEEDS, INC.</b>		2. TEMPORARY DESIGNATION OR EXPERIMENTAL NAME <b>6.L-230-1-M-M</b>		3. VARIETY NAME <b>BIG STAR</b>	
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code, and Country) <b>485 VICTOR WAY, SUITE 10 SALINAS, CA. 93907</b>		5. TELEPHONE (include area code) <b>(831) 757-0939</b>		FOR OFFICIAL USE ONLY PVPO NUMBER <b>200200013</b>	
6. FAX (include area code) <b>(831) 757-6829</b>		7. IF THE OWNER NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION (corporation, partnership, association, etc.) <b>CENTRAL VALLEY SEEDS, INC.</b>		8. IF INCORPORATED, GIVE STATE OF INCORPORATION <b>CALIFORNIA</b>	
9. DATE OF INCORPORATION <b>APRIL 14, 1987</b>		10. NAME AND ADDRESS OF OWNER REPRESENTATIVE(S) TO SERVE IN THIS APPLICATION. (First person listed will receive all papers) <b>M. MASSOUDI CENTRAL VALLEY SEEDS, INC. 485 VICTOR WAY, SUITE 10 SALINAS, CA. 93907</b>		FILING AND EXAMINATION FEES: \$ 2705.00 DATE <b>10/16/2007</b> CERTIFICATION FEE: \$ 682.00 DATE <b>9-20-05</b>	
11. TELEPHONE (include area code) <b>(831) 757 0939</b>		12. FAX (include area code) <b>(831) 757-6829</b>		13. E-MAIL <b>MMASSOUDI@CENTRAL VALLEY SEEDS.COM</b>	
14. CROP KIND (Common Name) <b>LACTUCA SATIVA</b>		15. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED (Follow instructions on reverse)			
<input checked="" type="checkbox"/> Exhibit A. Origin and Breeding History of the Variety <input checked="" type="checkbox"/> Exhibit B. Statement of Distinctness <input checked="" type="checkbox"/> Exhibit C. Objective Description of Variety <input checked="" type="checkbox"/> Exhibit D. Additional Description of the Variety (Optional) <input checked="" type="checkbox"/> Exhibit E. Statement of the Basis of the Owner's Ownership <input checked="" type="checkbox"/> Voucher Sample (2,500 viable untreated seeds or, for tuber propagated varieties, verification that tissue culture will be deposited and maintained in an approved public repository) <input checked="" type="checkbox"/> Filing and Examination Fee (\$2,705), made payable to "Treasurer of the United States" (Mail to the Plant Variety Protection Office)		16. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD AS A CLASS OF CERTIFIED SEED? See Section 83(a) of the Plant Variety Protection Act <input type="checkbox"/> YES (If "yes", answer items 20 and 21 below) <input checked="" type="checkbox"/> NO (If "no," go to item 22) 17. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO IF YES, WHICH CLASSES? <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED 18. DOES THE OWNER SPECIFY THAT THE CLASSES BE LIMITED AS TO NUMBER OF GENERATIONS? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO IF YES, SPECIFY THE NUMBER 1, 2, 3, etc. <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED (If additional explanation is necessary, please use the space indicated on the reverse.)			
22. HAS THE VARIETY (INCLUDING ANY HARVESTED MATERIAL) OR A HYBRID PRODUCED FROM THIS VARIETY BEEN SOLD, DISPOSED OF, TRANSFERRED, OR USED IN THE U. S. OR OTHER COUNTRIES? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. (Please use space indicated on reverse.)		23. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT (PLANT BREEDER'S RIGHT OR PATENT)? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO IF YES, GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. (Please use space indicated on reverse.)			
24. The owners declare that a viable sample of basic seed of the variety will be furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate. The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act. Owner(s) is(are) informed that false representation herein can jeopardize protection and result in penalties.					
SIGNATURE OF OWNER  NAME (Please print or type) <b>TONY M. AVILA</b>		SIGNATURE OF OWNER  NAME (Please print or type) <b>DAN AVILA</b>			
CAPACITY OR TITLE <b>VP SALES/RESEARCH</b>		DATE <b>10/12/2001</b>		CAPACITY OR TITLE <b>CFO</b>	
				DATE <b>10-12-01</b>	

**GENERAL:** To be effectively filed with the Plant Variety Protection Office (PVPO), **ALL** of the following items must be **received** in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to **reproduce** the variety, or for tuber reproduced varieties verification that a viable (in the sense that it will reproduce an entire plant) tissue culture will be deposited and maintained in an approved public repository; (4) check drawn on a U.S. bank for \$2,705 (\$320 filing fee and \$2,385 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice.) Partial applications will be held in the PVPO for not more than 90 days, then returned to the applicant as unfilled. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 500, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. **Retain one copy for your files.** All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. **DO NOT** use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amount of \$320 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

**Plant Variety Protection Office**

**Telephone: (301) 504-5518**

**FAX: (301) 504-5291**

**Homepage: <http://www.ams.usda.gov/science/pvp.htm>**

**ITEM**

- 18a. Give: (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;  
(2) the details of subsequent stages of selection and multiplication;  
(3) evidence of uniformity and stability; and  
(4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified
- 18b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:  
(1) identify these varieties and state all differences objectively;  
(2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and  
(3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 18c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 18d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, etc.
- 18e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
19. If "Yes" is specified (*seed of this variety be sold by variety name only, as a class of certified seed*), the applicant **MAY NOT** reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. (See Regulations and Rules of Practice, Section 97.103).
21. See Section 83 of the Act for the Contents and Term of Plant Variety Protection.
22. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
23. See Section 5.5 of the Act for instructions on claiming the benefit of an earlier filing date.

**21. CONTINUED FROM FRONT** (Please provide a statement as to the limitation and sequence of generations that may be certified.)

**22. CONTINUED FROM FRONT** (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)

17  
10-16-00 USA 2AD 7/13/05

**23. CONTINUED FROM FRONT** (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)

**NOTES:** It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. There is no charge for filing a change of address. The fee for filing a change of ownership or assignment or any modification of owner's name is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)

To avoid conflict with other variety names in use, the applicant must check the variety names proposed by contacting: Seed Branch, AMS, USDA, Room 213, Building 306, Beltsville Agricultural Research Center-East, Beltsville, MD 20705. Telephone: (301) 504-8089.

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number for this collection of information is (0581-0055). The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, political beliefs, sexual orientation, and marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact the USDA's TARGET Center at 202-720-2600 (voice and TDD). To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

S&T-470 (2-99) designed by the Plant Variety Protection Office with WordPerfect 6.0a. Replaces STD-470 (6-98) which is obsolete.

## Exhibit A

200200013

### Origin & Breeding History

'Big Star' (Experimental line G.L.-230-1-m-m) is a Cutting/Leaf type lettuce, *Lactuca sativa* L., variety. The breeding history is described below in detail and Figure 1 illustrates the pedigree and the invention history of Big Star.

In 1993, Big Star was instigated from a single green leaf/romaine outcross plant selected from a commercial Darkland Cos (PVP No. 9000137) field in Salinas Valley, California. The outcross plant did not show any tipburn symptoms and was non-cupping (closure of internal leaves) and displayed glossy dark green leaf color. The selected plant, labeled as *Canyon-1*, was allowed to self-pollinate in the greenhouse and the resulting seeds were collected. Single plant selection program was used to develop Big Star.

In 1994, approximately 50 seeds were planted in Salinas Valley, California. The materials at this stage were genetically segregating for the romaine/Cos type (~ 10-15%) and the green leaf type (~ 85-90%). A total of six single plant selections for the green leaf type were made for the next generation. The phenotypes of the selected plants were mostly alike except for minor differences such as the degree of leaf glossiness, green color, amount of leaf blistering, and open (non-cupping) to semi-open kind. Of six, only three plants survived. The plants were allowed to self pollinate and the resulting seeds were collected.

In 1995, approximately 50 seeds from each line were planted in Salinas Valley, California. Seven single plant selections were made from line *Canyon-1-3*. Selection was based on short core length, no tipburn symptoms, non-cupping with semi-glossy dark green leaf color and frilled leaf margins. Of seven, only six plants survived. The plants were allowed to self pollinate and the resulting seeds, segregating for seed color, were collected.

In 1996, seeds from each pedigree were planted in Salinas Valley, California. Plants of line *Canyon-1-3-2* were appeared to be genetically uniform for short core length, no tipburn symptoms, non-cupping with semi-glossy dark green leaf color and frilled leaf margins. Three individuals were selected and labeled as *Canyon-1-3-2-1*, 2 & 3. The plants were allowed to self pollinate in the greenhouse and the resulting seeds were collected.

In 1997, approximately 50 seeds from each line were planted in Gilroy, California for tipburn resistance and the described phenotypic assessments. Plants of line *Canyon-1-3-2-2* were appeared to be genetically uniform for tipburn resistance, short core length, non-cupping with semi-glossy dark green leaf color and frilled leaf margins. Genetic stability was prominent in this F<sub>5</sub> generation. Five individuals were selected from *Canyon-1-3-2-2* and were labeled accordingly. Of five, only three plants survived and they were allowed to self-pollinate in the greenhouse and the resulting seeds were collected.

200200013

In 1998, seeds from each line were planted in Gilroy, California. Plants from each line appeared to be genetically stable and uniform for tipburn resistance, short core length, non-spiral, non-cupping with semi-glossy dark green leaf color, and frilled leaf margins. Five individual plant selections were selected from line *Canyon-1-3-2-2-3*. Plants were allowed to self pollinate in the greenhouse and the resulting seeds were collected.

In 1999, one hundred twenty four seeds from each line were greenhouse germinated and were later transplanted in Sanger, California. Plants of lines *Canyon-1-3-2-2-3-1* through -5 were uniform and appeared to be genetically stable. Preference was given to line *Canyon-1-3-2-2-3-1* and the entire line was collectively massed in the seed production field and the resulting seeds were collected.

In October of 1999, seeds of line *Canyon-1-3-2-2-3-1* were planted in a 50 ft. x 100 ft. insect resistance seed cage in Winterheaven, California. All plants were appeared to be genetically and phenotypically uniform in accordance to our selection criteria. At this stage, the experimental name *Canyon-1-3-2-2-3-1-mass* was changed to *G.L.-230-1-m*.

In year 2000, the experimental line *G.L.-230-1-m* was massed increased in Central Valley Seeds' commercial seed production fields in Five Points, California. The experimental designated breeding line *G.L.-230-1-m-m* was given the name 'Big Star'. The field performance, adaptability and type acceptability of 'Big Star' has been examined and confirmed in several lettuce growing areas of California and Arizona. Compared to other available commercial green leaf varieties under higher than normal temperatures and adverse environmental conditions, 'Big Star' does not spiral, stays open and is non-cupping (non-heading), is highly resistant to tipburn and is very pliable for packaging and shipping. Further more, our field observations suggest that 'Big Star' is harvestable and does not break down and holds its phenotypic characteristics 10-14 days passed its optimum maturity harvest date. Based on our field and commercial seed production trials, it has been determined that the green leaf lettuce variety 'Big Star' is genetically distinct, stable and uniform and no variants or off types have been observed.

**Exhibit A: Origin & Breeding Statement:**

200200013

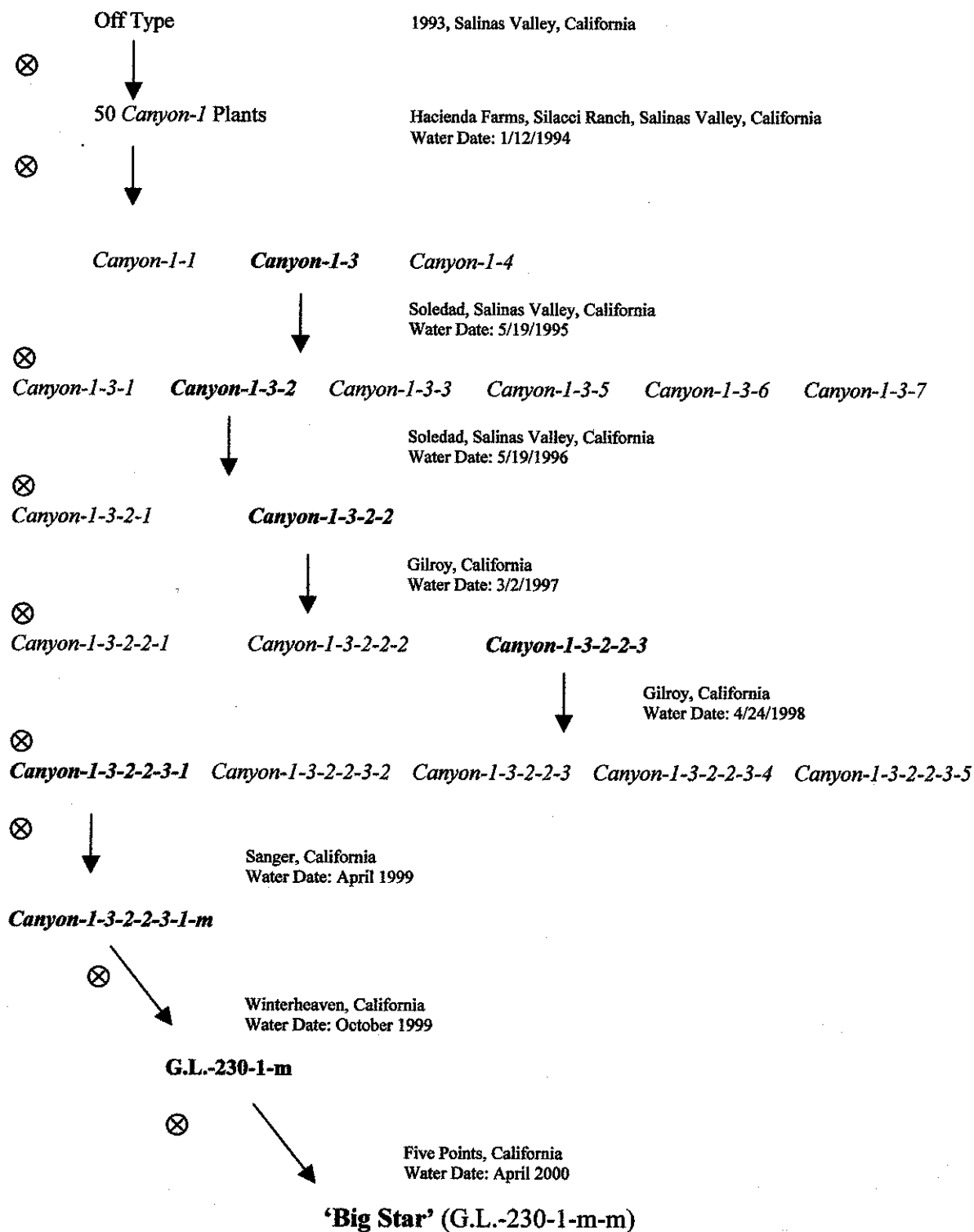
As stated in the PVP application, Big Star was originated from a single green leaf/romaine outcross plant selected from a commercial Darkland Cos (PVP No. 9000137) field in Salinas Valley, California. We could not locate any records for the 1991 and 1992 seed production years to possibly identify and locate any green leaf type variety growing adjacent or nearby the "Darkland Cos" lettuce. However, in the early 1990's Central Valley Seeds did produce the green leaf lettuce "Hacienda" and "Crisp & Green" as a commercial varieties. We cannot be certain but it is possible that any of the named green leaf lettuce varieties might have been the pollinated source for the original outcross. Finding such genetic outcrosses in the commercial fields is not totally uncommon. For example, lettuce variety Two Star (Plant Variety Protection Number 9200270) was originally developed from an off type of romaine lettuce variety Green Towers.

In the commercial Darkland Cos (PVP No. 9000137) planting field in Salinas Valley, California where the off type was found, there were only a couple of plant individuals that were noted to be different. However, only one plant was selected and developed into 'Big Star'.

The seed color of lettuce variety 'Hacienda' is black.

"Big Star" has been observed for seven generations of reproduction and during the seed increase period and is stable and uniform. No variants were observed.

**Figure 1. The Pedigree of Lettuce, *Lactuca sativa*, Green Leaf variety 'Big Star'**



**Exhibit B****Additional Novelty Statement****Statistical Analysis**

Measurable characteristics were assessed in several localities or dates and the results were analyzed separately. Unless otherwise indicated, the statistical analyses were performed using T-test. The results presented in actual t-value and probability values  $p[t]$ . The standard of deviation for each variety in the comparisons is presented in sigma value. LSD mean 95% corresponds to Least Significant Difference between the means at the 95% probability level.

**Statement Of Distinctness**

'Big Star' belongs to the Cutting/Leaf type lettuce, *Lactuca sativa* L. varieties. 'Big Star' is adapted for the lettuce growing regions of California and Arizona. 'Big Star' is unique for its class and is relatively medium in height, with a medium frame structure, very thick and slightly savoy (blistering) leaf texture, slight to moderate leaf undulation, semi-glossy green leaf color, short core length, and a flat butt shape with smooth midribs. 'Big Star' expresses resistance to tipburn and does not spiral (twisting of the plant at maturity). According to the Munsell Color Chart for Plant Tissues, 'Big Star' has leaf color Value 4 Chroma 6 Hue 5 GY. 'Big Star' seed color is white. 'Big Star' is genetically pure and stable and has excellent seed emergence.

RAD 8/17/05  
moderately raised

**Example # 1**

'Big Star' is similar to 'Two Star'; however,

Area	'Big Star'	'Two Star'
Seed Color	White	Black
Leaf Blistering (Savoyedness)	Slight moderate	Moderate Strong
Plant Height	Medium	Tall
Maturity	About 2-3 Days Late	About 2-3 Days Early
Tipburn	Highly Resistant	Intermediate
Leaf Undulation	Intermediate moderate	Severe Strong
Cupping	Non-cupping	Intermediate
Outer (Older) Leaf Shape	Wide at Bottom	Narrow at Bottom

RAD  
7/27/05

**NOTE:** As an experimental variety, 'Big Star' was consistently evaluated at the harvest maturity date of 'Two Star', which is in average 2 to 3 days earlier in harvest maturity than 'Big Star'. Therefore at its optimum harvest maturity, 'Big Star' will be heavier in an average of 50-60 grams in weight. Furthermore, 'Big Star' is harvestable 7-10 days passed its optimum maturity date.

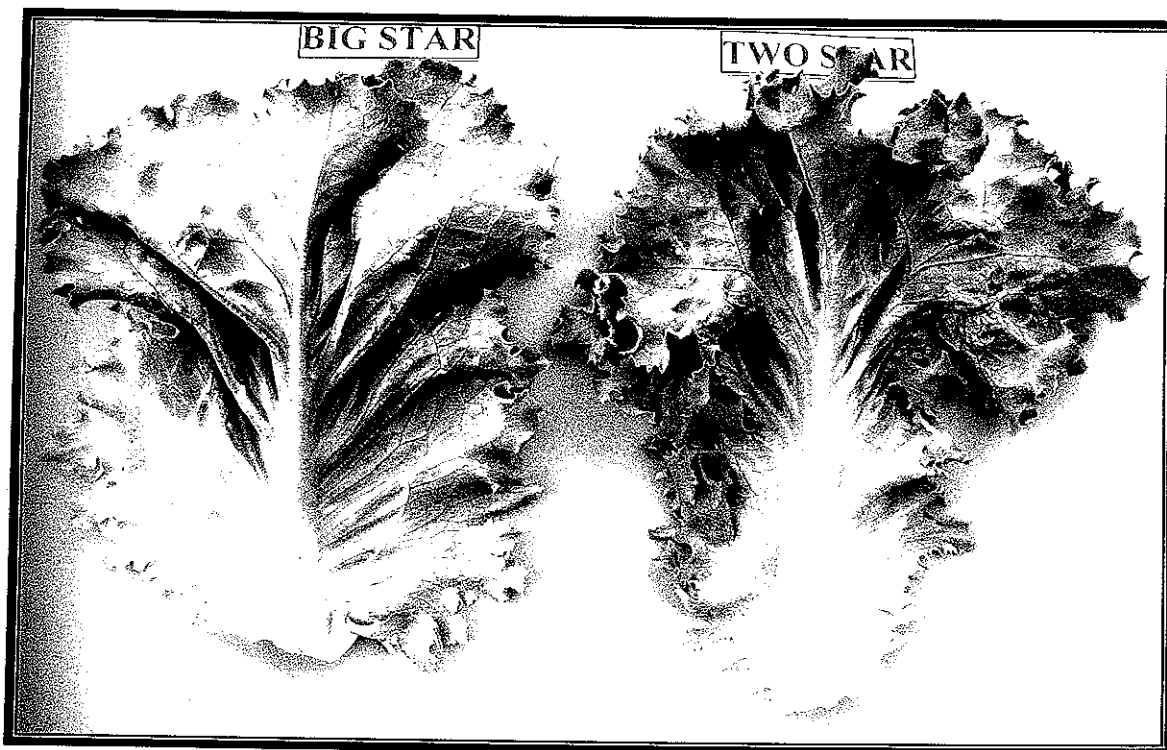
**Example #2**

'Big Star' is most similar to 'Two Star'; however, 'Big Star' is significantly different than 'Two Star' based on the following measurements:



**Exhibit D**

Close-up Top & Under Side Pictures of 'Big Star' and 'Two Star' Mature Leaves at Maturity Stage



Source: Duncan Ranch, Gabilan View. Salinas, California, Lot # 13. Water date: 4/18/2001. Photographs taken: 6/12/2001.

Central Valley Seeds, Inc.

AGRICULTURAL MARKETING SERVICE  
LIVESTOCK AND SEED DIVISION  
OBJECTIVE DESCRIPTION OF VARIETY  
LETTUCE *Lactuca sativa*

EXHIBIT C

NAME OF APPLICANT (S) <b>CENTRAL VALLEY SEEDS, INC.</b>	FOR OFFICIAL USE ONLY PVPO NUMBER <b>200200013</b>
ADDRESS (Street and No. or R.F.D. No., City, State, and ZIP Code) <b>485 VICTOR WAY, SUITE 10 SALINAS, CA. 93907</b>	VARIETY NAME <b>BIG STAR</b> EXPERIMENTAL DESIGNATION <b>G.L. - 230-1-M-M</b>

Place numbers in the boxes for the characters which best describe this variety. Measured data should be the mean of an appropriate number (at least 10) of well spaced plants. Royal Horticultural Society or any recognized color standard may be used to determine plant colors.

The location of the test area is: **SALINAS, CALIFORNIA** Color System Used: **MUNSELL COLOR CHART FOR PLANT TISSUE**

PLANT TYPE: (See list of suggested check varieties page 4.)

☒ 01

01=Cutting/Leaf  
02=Butterhead  
03=Bibb  
04=Cos or Romaine

05=Great Lakes Group  
06=Vanguard Group  
07=Imperial Group  
08=Eastern (Ithaca) Group

09=Stem  
10=Latin  
11=OTHER

SEED:

COLOR

LIGHT DORMANCY

HEAT DORMANCY

☒ 1

1=White (Silver Gray)  
2=Black (Gray Brown)  
3=Brown (Amber)

☒ 1

1=Light Required  
2=Light Not Required

☒ 1

1=Susceptible  
2=Not Susceptible

COTYLEDON TO FOURTH LEAF STAGE: NOTE: Provide a color photograph or photocopy of the fourth leaf from 20 day old seedling grown under optimal conditions.

☒ 2

SHAPE OF COTYLEDONS:

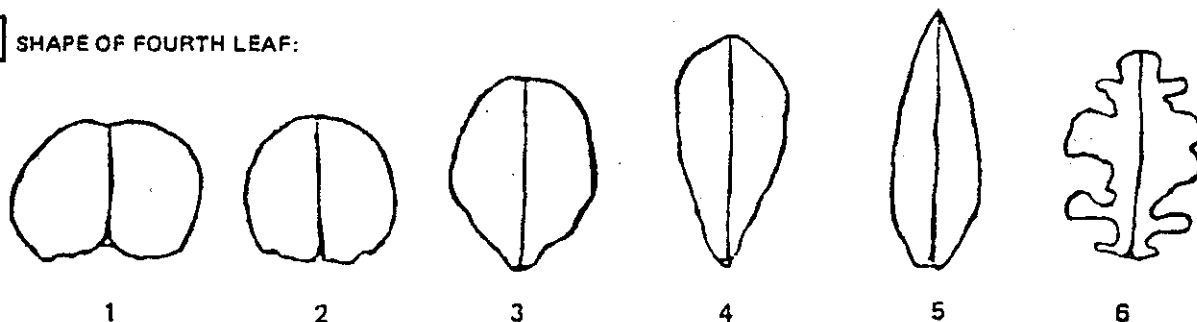
1=Broad

2=Intermediate

3=Spatulate

☒ 3

SHAPE OF FOURTH LEAF:



☐ ☐

LENGTH/WIDTH INDEX OF FOURTH LEAF: L/W x 10

☒ 2

APICAL MARGIN:

1=Entire  
2=Crenate/Gnawed  
3=Finely Dentate

4=Moderately Dentate  
5=Coarsely Dentate  
6=Incised

7=Lobed  
8=OTHER (specify)

☒ 2

BASAL MARGIN:

☒ 2

UNDULATION:

1=Flat

2=Slight

3=Medium

4=Marked

☒ 4

GREEN COLOR:

1=Yellow Green  
2=Light Green

3=Medium Green  
4=Dark Green

5=Blue Green  
6=Silver Green

7=Gray Green

ANTHOCYANIN:

☒ 1

DISTRIBUTION:

1=Absent  
2=Margin Only

3=Spotted  
4=Throughout

5=OTHER (specify)

☐

CONCENTRATION:

1=Light

2=Moderate

3=Intense

☒ 2

ROLLING:

1=Absent

2=Present

☒ 2

CUPPING:

1=Uncupped

2=Slight

3=Markedly

☒ 1

REFLEXING:

1=None

2=Apical Margin

3=Lateral Margins

#### 4. MATURE LEAVES (observe harvest-mature outer leaves):

NOTE: Provide color photo of harvest-mature leaves which accurately shows color and margin characteristics.

200200013

##### MARGIN:

2	INCISION DEPTH: (deepest penetration of the margin)	1=Absent/Shallow (Dark Green Boston)	2=Moderate (Vanguard)	3=Deep (Great Lakes 659)
2	INDENTATION: (finest divisions of the margin)	1=Entire (Dark Green Boston)	3=Deeply Dentate (Great Lakes 659)	5=OTHER (specify)
		2=Shallowly Dentate (Great Lakes 65)	4=Crenate (Vanguard)	
2	UNDULATION OF THE APICAL MARGIN:	1=Absent/Slight (Dark Green Boston)	2=Moderate (Vanguard)	3=Strong (Great Lakes 659)
3	GREEN COLOR:	1=Very Light Green (Bibb)	3=Medium Green (Great Lakes)	5=Very Dark Green
		2=Light Green (Minetto)	4=Dark Green (Vanguard)	6=OTHER
ANTHOCYANIN (grown at or below 10 C):				
1	DISTRIBUTION:	1=Absent	3=Spotted (Calif. Cream Butter)	5=OTHER (specify)
		2=Margin Only (Big Boston)	4=Throughout (Prize Head)	
	CONCENTRATION:	1=Light (Iceberg)	2=Moderate (Prize Head)	3=Intense (Ruby)
2	SIZE:	1=Small	2=Medium	3=Large
2	GLOSSINESS:	1=Dull (Vanguard)	2=Moderate (Salinas)	3=Glossy (Great Lakes)
2	BLISTERING:	1=Absent/Slight (Salinas)	2=Moderate (Vanguard)	3=Strong (Prize Head)
3	LEAF THICKNESS:	1=Thin	2=Intermediate	3=Thick
1	TRICHOMES:	1=Absent (smooth)	2=Present (spiny)	

#### 5. PLANT (at market stage. Choose a comparison variety appropriate for this type.):

##### SPREAD OF FRAME LEAVES:

20' cm This Variety 25' cm (specify comparison variety)

##### HEAD DIAMETER (market trimmed with single cap leaf):

cm This Variety cm (specify comparison variety)

5 HEAD SHAPE: 1=Flattened 2=Slightly Flattened 3=Spherical 4=Elongate 5=Non-Heading 6=OTHER

2 HEAD SIZE CLASS: 1=Small 2=Medium 3=Large

24 HEAD COUNT PER CARTON

##### HEAD WEIGHT:

454 g This Variety 524 g 2-STAR (specify comparison variety)

1 HEAD FIRMNESS: 1=Loose 2=Moderate 3=Firm 4=Very Firm

#### 6. BUTT (bottom of market-trimmed head):

2 SHAPE: 1=Slightly Concave 2=Flat 3=Rounded

2 MIDRIB: 1=Flattened (Salinas) 2=Moderately Raised 3=Prominently Raised (Great Lakes 659)

#### 7. CORE (stem of market-trimmed head):

3.0 mm Diameter at base of head TWO STAR 33

##### Ratio of head diameter/core diameter

##### Core height from base of head to apex:

47 mm This Variety 45 mm 2 STAR (specify comparison variety)

#### 8. BOLTING (Give First Water Date):

NOTE: First Water Date is the date seed first receives adequate moisture to germinate. This can and often does equal the planting date.

##### Number of days from First Water Date to seed stalk emergence (summer conditions):

59 This Variety 510 2 STAR (specify comparison variety)

2 BOLTING CLASS: 1=Very Slow 2=Slow 3=Medium 4=Rapid 5=Very Rapid

##### Height of mature seed stalk:

101 cm This Variety 120 cm TWO STAR (specify comparison variety)

8. BOLLING cont'd.

Spread of Bolter Plant (at widest point):  
 42 cm This Variety 53 cm TWO STAR (specify comparison variety) 200200013

2 BOLTER LEAVES: 1=Straight 2=Curved

2 MARGIN: 1=Entire 2=Dentate

2 COLOR: 1=Light Green 2=Medium Green 3=Dark Green

BOLTER HABIT:

2 TERMINAL INFLORESCENCE: 1=Absent 2=Present

1 LATERAL SHOOTS: (above head) 1=Absent 2=Present

1 BASAL SIDE SHOOTS: 1=Absent 2=Present

9. MATURITY (earliness of harvest-mature head formation):

NOTE: Complete this section for at least one season.

SEASON	Applic. 1/ #of days	Check 2/ #of days	CHECK VARIETY 2/
Spring	71	68	TWO STAR
Summer	59	56	TWO STAR
Fall	68	65	TWO STAR
Winter			

Give planting date(s), and location(s):

Spring MARCH 16, 2001 SALINAS, CALIFORNIA

Summer APRIL 18, 2001 SALINAS, CALIFORNIA

Fall AUGUST 7, 2001 GONZALES, CALIFORNIA

Winter

1/ First water date to harvest.

2/ Fill in check variety name on the appropriate line.

10. ADAPTATION:

PRIMARY REGIONS OF ADAPTION (tested and proven adapted):

(0=Not tested

1=Not Adapted

2=Adapted)

2 Southwest (Calif., Ariz. desert) 2 West Coast 0 Northeast

0 Northcentral 0 Southeast OTHER

SEASON:

2 Spring (area WEST COAST) 2 Fall (area WEST COAST)

2 Summer (area WEST COAST) 2 Winter (area CA, AZ DESERT)

0 GREENHOUSE: 0=Not tested 1=Not Adapted 2=Adapted

3 SOIL TYPE: 1=Mineral 2=Organic 3=Both

11. DISEASES AND STRESS REACTIONS (0=Not tested; 1=Susceptible; 2=Intermediate; 3=Resistant; 4=Highly resistant; 5=Tolerant):

VIRUS

- ☒ 2 Big Vein  
☐ 1 Lettuce Mosaic  
☐ 0 Cucumber Mosaic  
☐ 0 Broad Bean Wilt  
☐ 0 Turnip Mosaic  
☐ 0 Beet Western Yellows  
☐ 0 Lett. Infectious Yellows  
☐ 0 Other Virus \_\_\_\_\_

FUNGAL/BACTERIAL

- ☐ 0 Corky Root Rot (Pythium Root Rot)  
☐ 0 Downy Mildew (Races \_\_\_\_\_)  
☐ 0 Powdery Mildew  
☐ 0 Sclerotinia Rot  
☐ 0 Bacterial Soft Rot (Pseudomonas spp. & others)  
☐ 0 Botrytis (Gray Mold)  
☐ 0 OTHER \_\_\_\_\_

INSECTS

- ☐ 0 Cabbage Loopers  
☐ 0 Root Aphids  
☐ 0 Green Peach Aphid  
☐ 0 Other Insect \_\_\_\_\_

PHYSIOLOGICAL/STRESS

- ☒ 3 Tipburn  
☐ 0 Heat  
☐ 0 Drought  
☐ 0 Cold  
☐ 0 Salt  
☐ 0 Brown Rib (Rib Discoloration, Rib Blight)  
☐ 0 OTHER \_\_\_\_\_

POST HARVEST

- ☐ 0 Pink Rib  
☐ 0 Russet Spotting  
☐ 0 Rusty Brown Discoloration  
☐ 0 Internal Rib Necrosis (Blackheart, Gray Rib, Gray Streak)  
☐ 0 Brown Stain

12. BIOCHEMICAL OR ELECTROPHORETIC MARKERS:

13. COMMENTS:

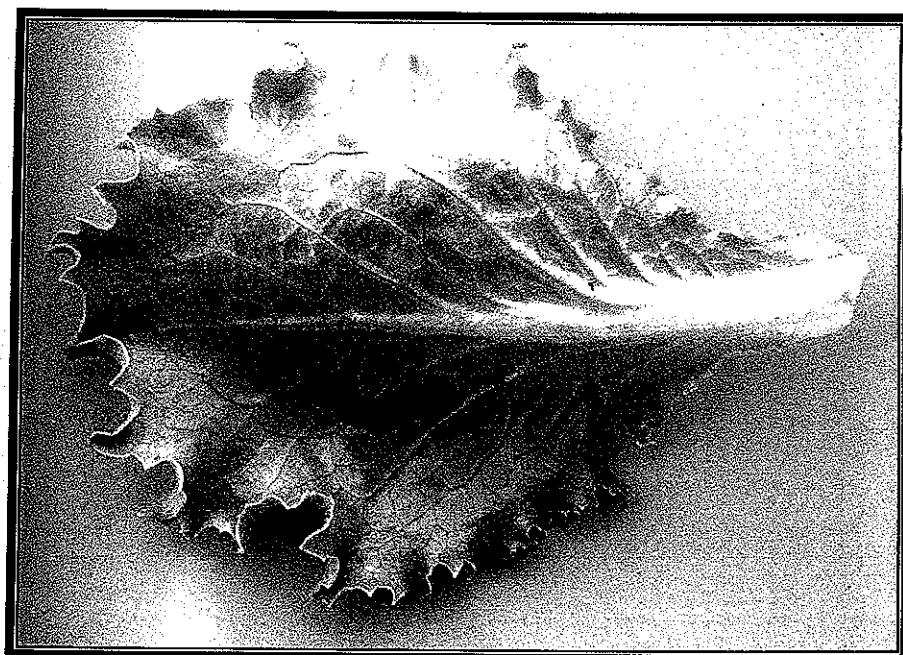
SUGGESTED CHECK VARIETIES

- |     | <u>TYPE</u>       |
|-----|-------------------|
| 1)  | CUTTING/LEAF      |
| 2)  | BUTTERHEAD        |
| 3)  | BIBB              |
| 4)  | COS, OR ROMAINE   |
| 5)  | GREAT LAKES GROUP |
| 6)  | VANGUARD GROUP    |
| 7)  | IMPERIAL GROUP    |
| 8)  | EASTERN GROUP     |
| 9)  | STEM              |
| 10) | LATIN             |

- | <u>CHECK VARIETY</u> |
|----------------------|
| SALAD BOWL           |
| DARK GREEN BOSTON    |
| BIBB                 |
| PARRIS ISLAND        |
| GREAT LAKES 659-700  |
| VANGUARD             |
| VIVA                 |
| ITHACA               |
| CELTUCE              |
| MATCHLESS            |

Exhibit D  
Additional Description of 'Big Star'

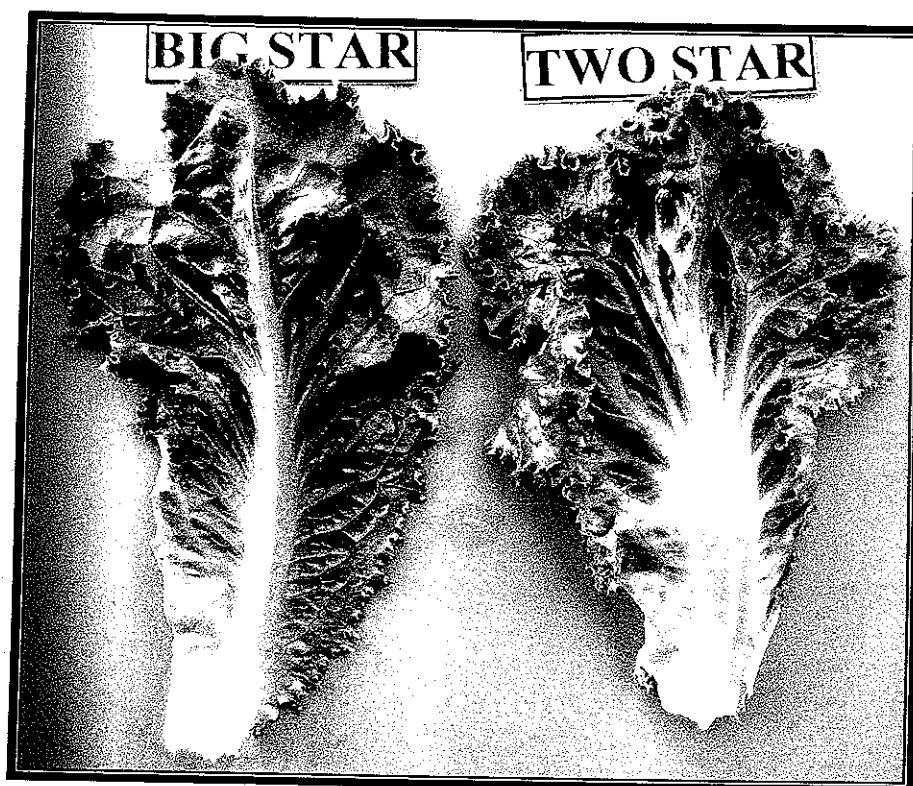
Top and Under Side Pictures of 'Big Star' Cotyledon at Fourth Leaf Stage



**Exhibit D**

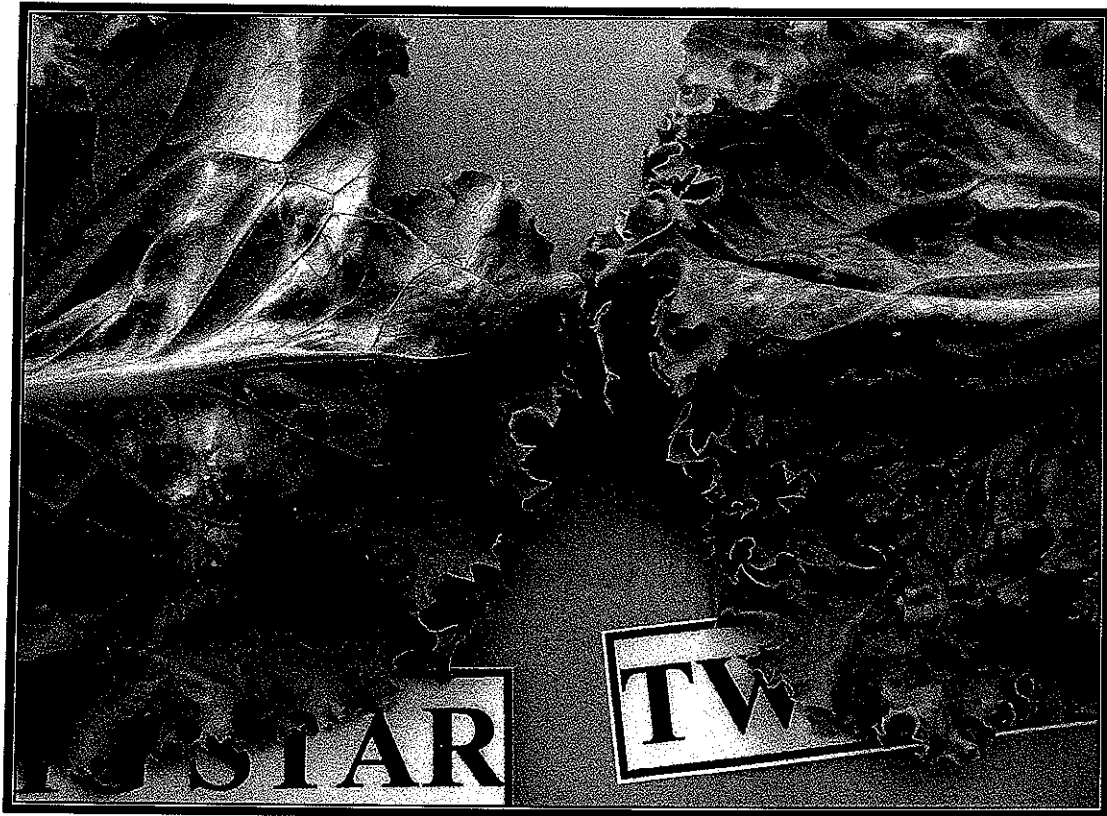
200200013

**Close-up Top & Under Side Pictures of 'Big Star' and 'Two Star' Interior (Younger)  
Leaves at Maturity Stage**



Source: Duncan Ranch, Gabilan View. Salinas, California, Lot # 13. Water date: 4/18/2001. Photographs taken: 6/12/2001.

Central Valley Seeds, Inc.

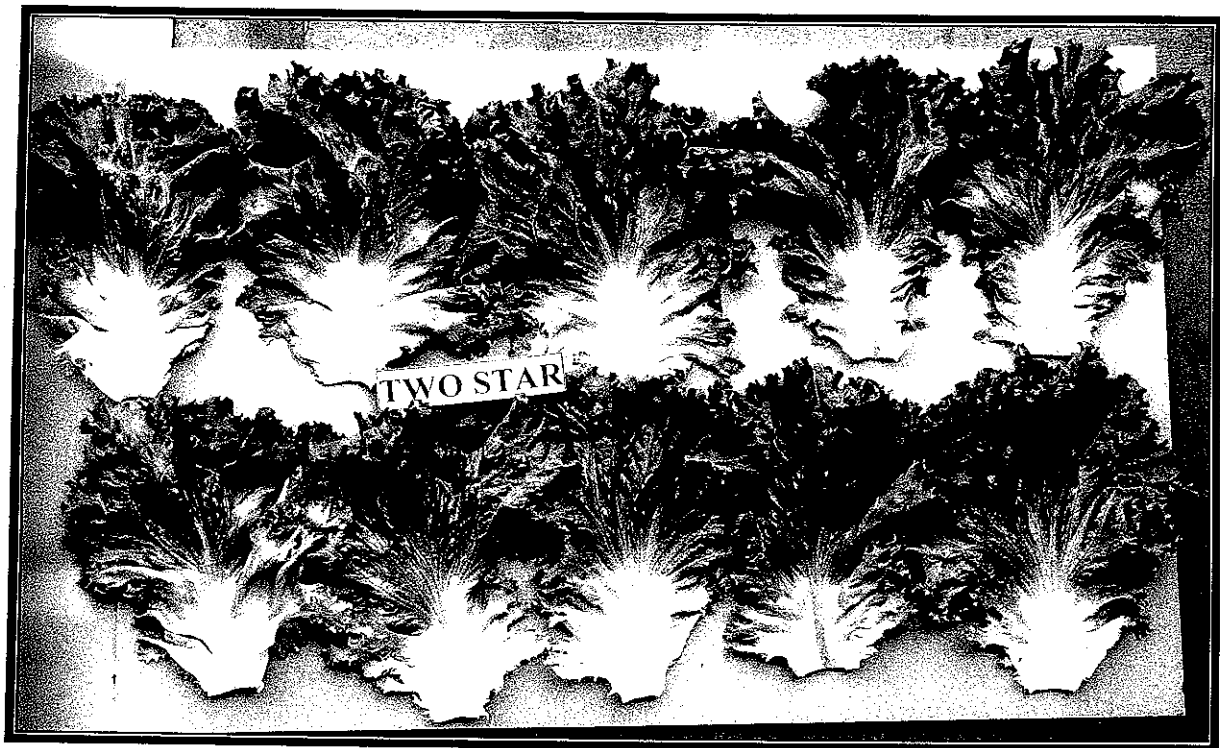
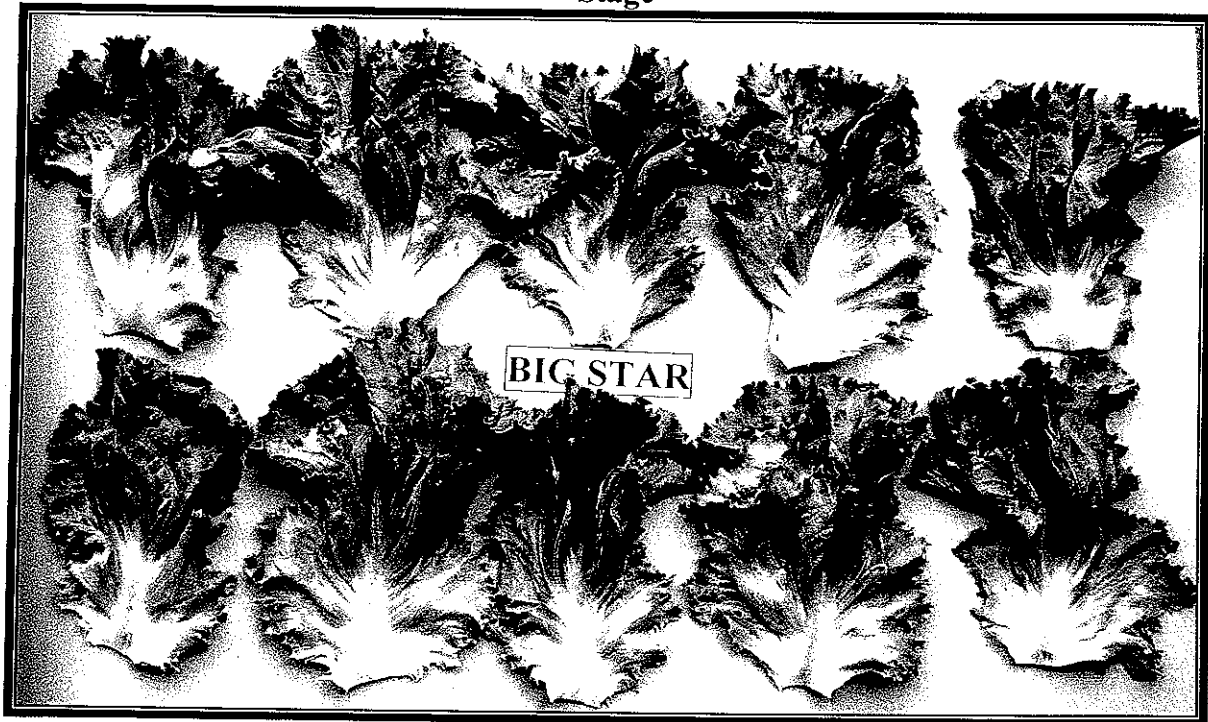
**Exhibit D****Close-up Leaf Margin Picture of 'Big Star' and 'Two Star' Interior (Younger) Leaves at Maturity Stage****'Big Star'****'Two Star'**

Source: Duncan Ranch, Gabilan View. Salinas, California, Lot # 13. Water date: 4/18/2001. Photographs taken: 6/12/2001.



# Exhibit D

Top Side Pictures of 'Big Star' and 'Two Star' Leaves of Ten individual Plants at Maturity Stage

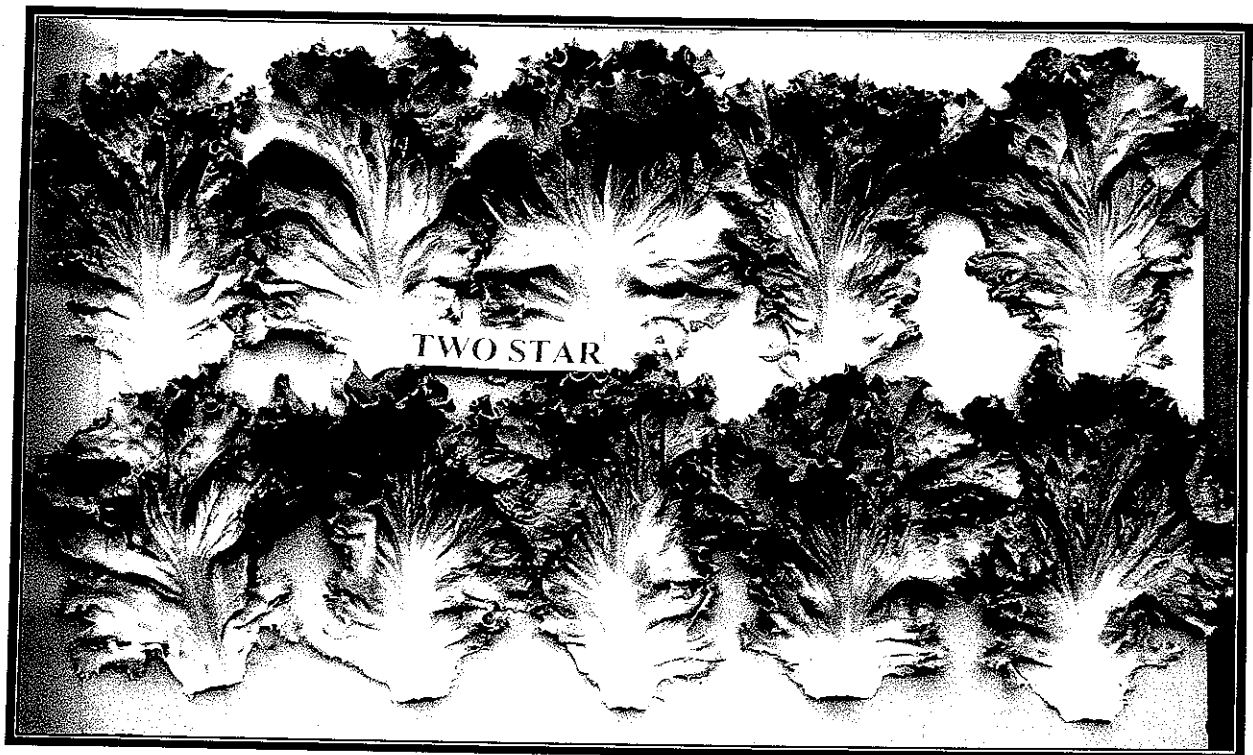
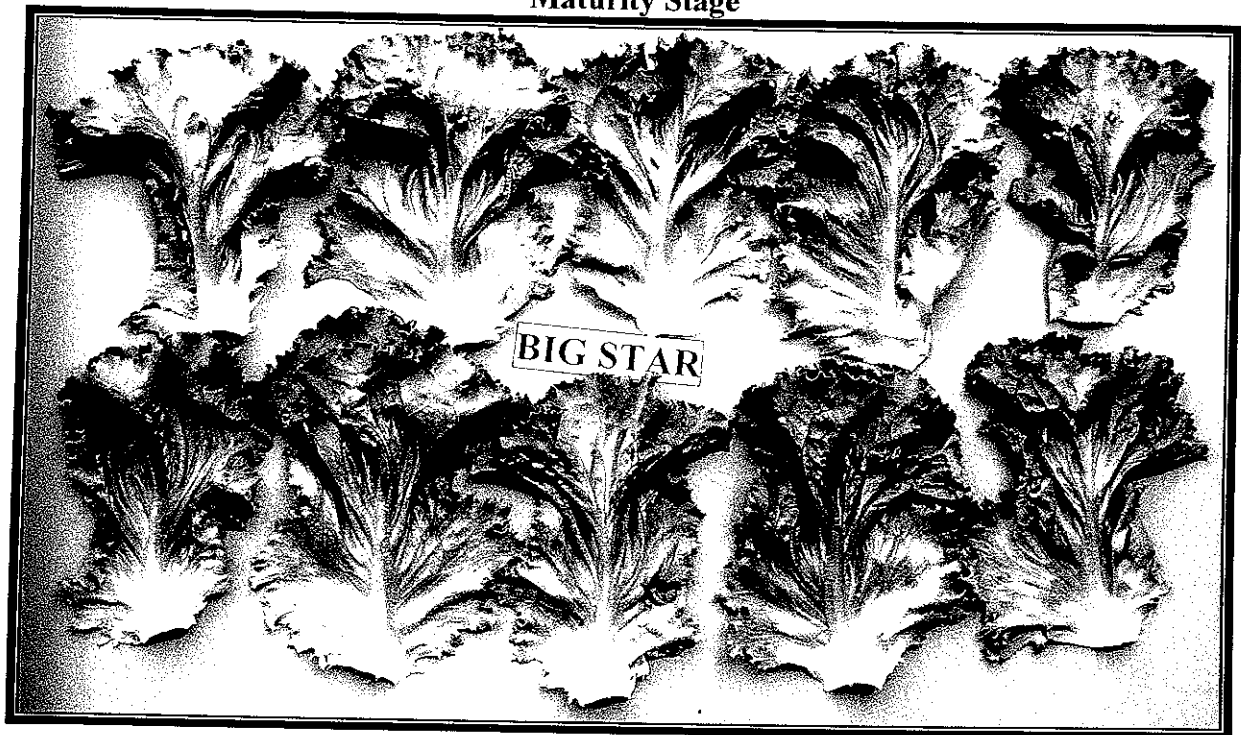


Source: D' Arrigo Bros. Company. Salinas, California, Ranch # 15, Lot # 29. Water date: 3/16/2001. Photographs taken: 5/22/2001

260205013

**Exhibit D**

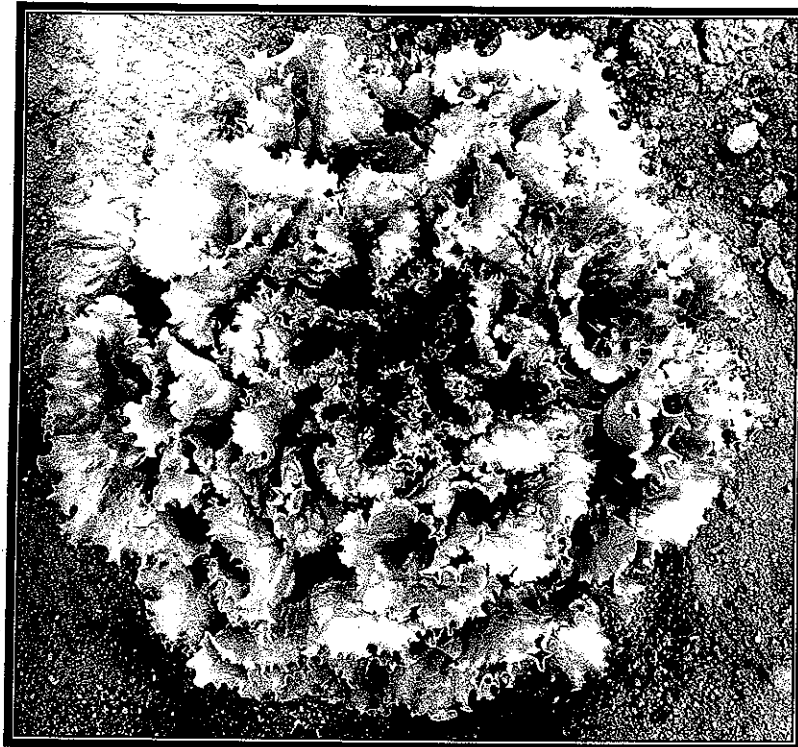
**Under Side Pictures of 'Big Star' and 'Two Star' Leaves of Ten individual Plants at Maturity Stage**



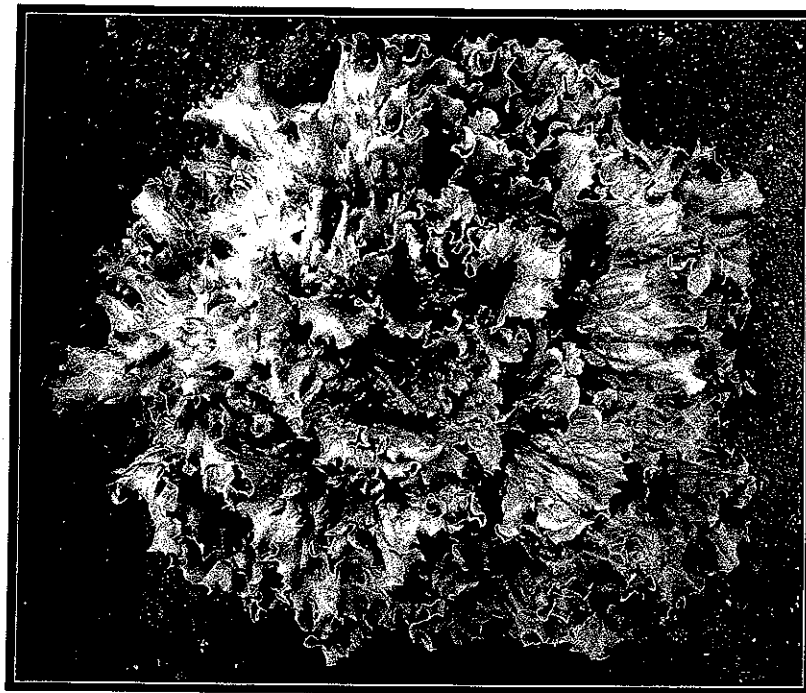
Source: D' Arrigo Bros. Company. Salinas, California, Ranch # 15, Lot # 29. Water date: 3/16/2001. Photographs taken: 5/22/2001

## Exhibit D

### 'Big Star' and 'Two Star' Appearances at Maturity Stage



'Big Star'



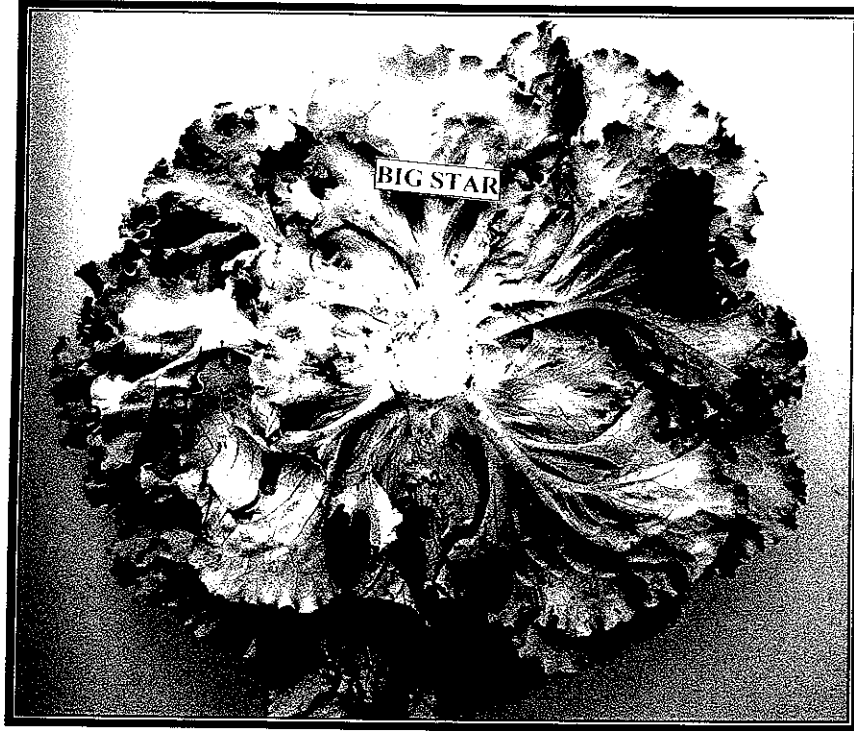
'Two Star'

Source: D' Arrigo Bros. Company. Salinas, California, Ranch # 15, Lot # 29. Water date: 3/16/2001. Photographs taken: 5/22/2001

Central Valley Seeds, Inc.

## Exhibit D

### 'Big Star' and 'Two Star' Butt Appearances at Maturity Stage



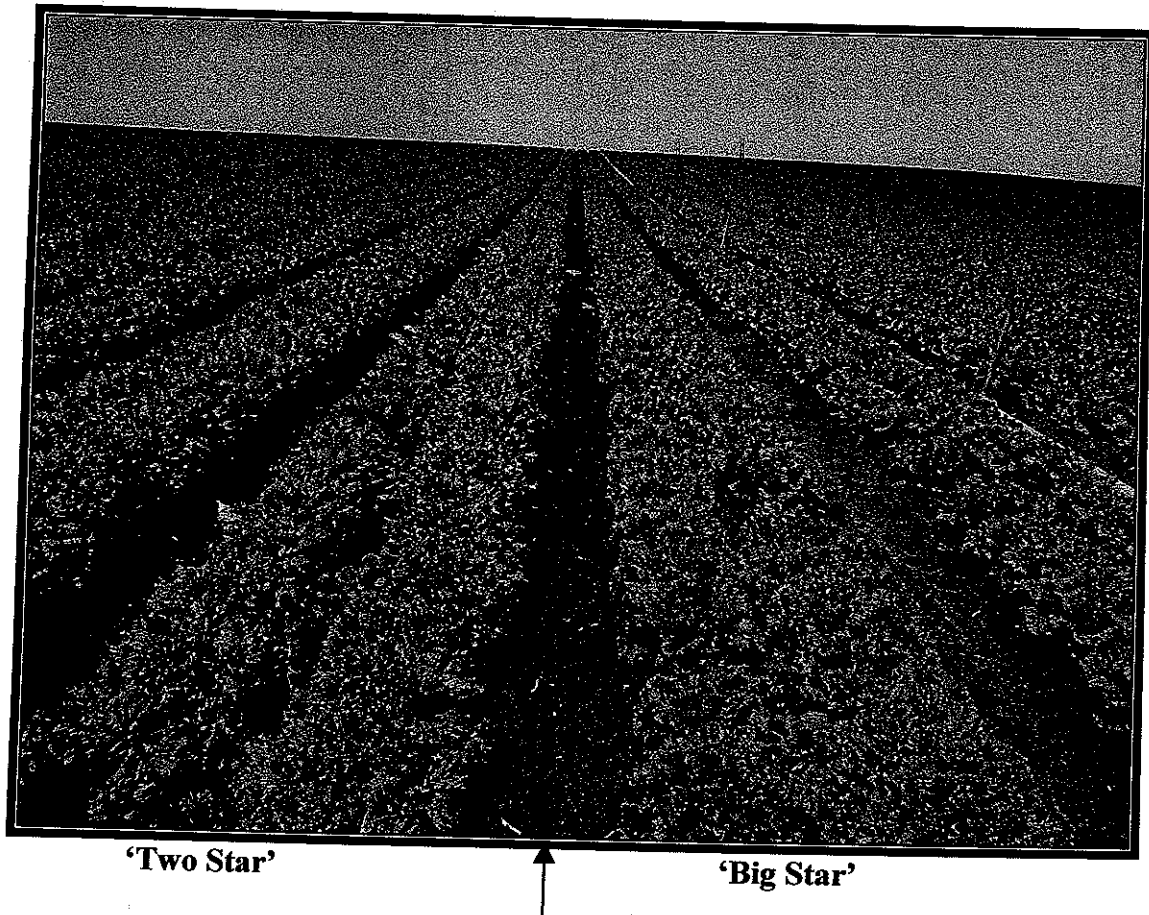
'Big Star'



'Two Star'

Source: D' Arrigo Bros. Company. Salinas, California, Ranch # 15, Lot # 29. Water date: 3/16/2001. Photographs taken: 5/22/2001

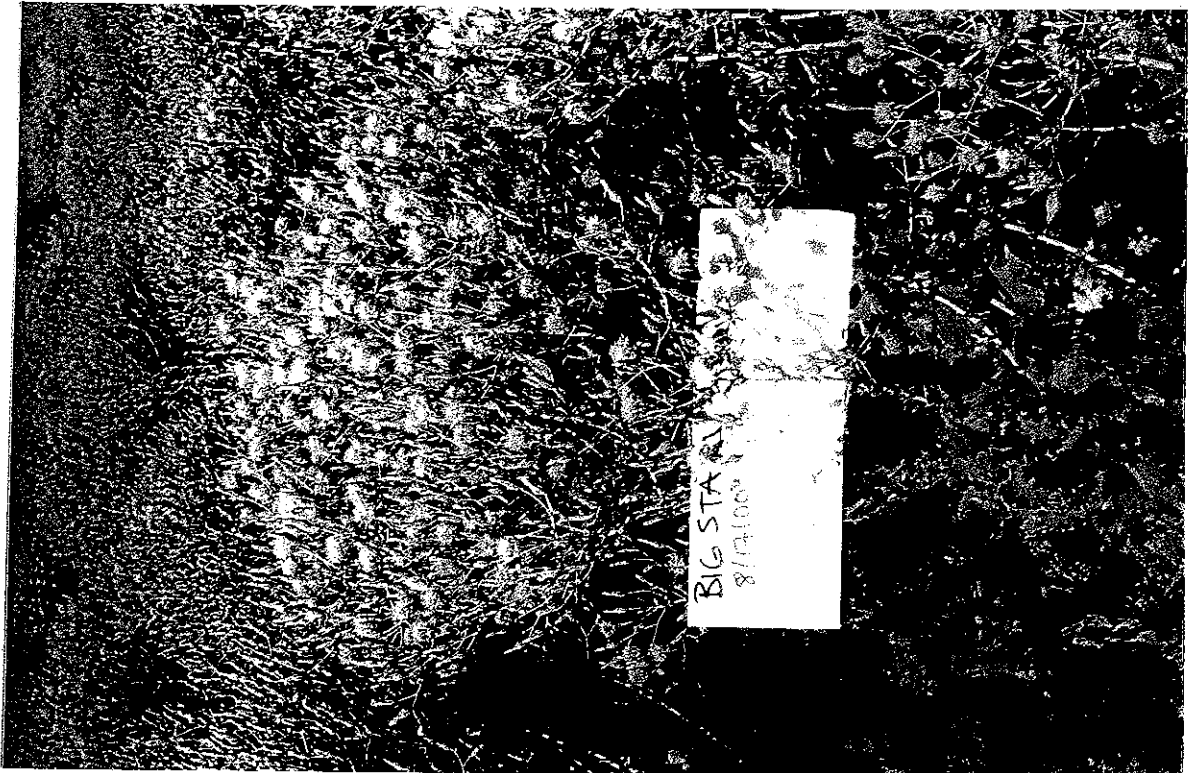
Commercial Field Production of 'Big Star' and 'Two Star'



Source: D' Arrigo Bros. Company. Salinas, California, Ranch # 5. Water date: 3/16/2001. Photographs taken: 6/13/2001.

**Exhibit D**

Photos of 'Big Star' at Seed Stage. Central Valley Seeds, Inc. Commercial Seed Production Field, Five Points, California. Photos taken 8/7/2000.





Variable	Trial Location	No. Plants/Rep	Rep No.	t*-value	p[t*]	Avg. 'Big Star'	Avg. 'Two Star'	LSD Mean 95%	Sigma
Plant Weight (g)	1	10	1	2.879	0.014	517	592	58.24	61.99
	1	10	2	5.093	0.000	458	562	45.67	48.60
	2	10	1	2.591	0.026	429	490	52.64	56.02
	2	10	2	2.569	0.027	419	454	30.47	32.43
	3	10	1	3.395	0.005	492	590	64.55	68.70
	3	10	2	2.376	0.039	513	582	64.93	69.11
	4	10	1	2.737	0.019	377	427	40.85	43.47
	4	10	2	4.240	0.001	363	423	31.65	33.68
Core Diameter (cm)	1	10	1	4.119	0.001	3.21	3.42	0.11	0.12
	1	10	2	3.604	0.003	3.16	3.38	0.14	0.15
	2	10	1	3.921	0.002	3.03	3.27	0.14	0.15
	2	10	2	3.670	0.003	3.01	3.26	0.15	0.16
	3	10	1	2.662	0.022	3.27	3.45	0.15	0.16
	3	10	2	2.800	0.017	3.30	3.50	0.16	0.17
	4	10	1	2.625	0.024	3.24	3.09	0.13	0.14
	4	10	2	2.487	0.031	3.23	3.07	0.14	0.15
Leaf Length (cm)	1	10	1	6.948	0.000	18.73	21.45	0.88	0.93
	1	10	2	6.287	0.000	18.86	21.96	1.10	1.17
	2	10	1	3.604	0.003	18.85	20.97	1.32	1.40
	2	10	2	5.202	0.000	18.11	21.82	1.59	1.70
	3	10	1	7.004	0.000	18.15	21.50	1.07	1.14
	3	10	2	7.273	0.000	18.36	21.26	0.89	0.95
	4	10	1	3.282	0.006	19.89	21.78	1.29	1.37
	4	10	2	4.025	0.001	19.26	21.70	1.36	1.44
Leaf Width (cm)	1	10	1	6.169	0.000	22.57	26.96	1.59	1.69
	1	10	2	6.087	0.000	20.32	25.96	2.07	2.21
	2	10	1	4.881	0.000	20.74	23.56	1.29	1.37
	2	10	2	2.871	0.015	20.94	22.84	1.48	1.57
	3	10	1	6.926	0.000	19.53	26.50	2.25	2.39
	3	10	2	7.688	0.000	20.62	25.86	1.52	1.62
	4	10	1	9.088	0.000	19.70	24.98	1.30	1.38
	4	10	2	12.246	0.000	18.93	24.91	1.09	1.16
<sup>a</sup> Leaf area coefficient	1	10	1	9.597	0.000	421	578	36.68	39.04
	1	10	2	8.835	0.000	382	570	47.51	50.56
	2	10	1	6.793	0.000	390	493	33.79	35.96
	2	10	2	5.939	0.000	378	498	45.00	47.90
	3	10	1	8.291	0.000	354	570	58.16	61.90
	3	10	2	8.810	0.000	379	550	43.46	46.25
	4	10	1	6.920	0.000	393	544	49.04	52.19
	4	10	2	9.944	0.000	364	540	39.60	42.14

## Field Trial Locations:

1. D' Arrigo Bros. Company. Salinas, California, Ranch # 15, Lot # 29. Water date: 3/16/2001, Evaluation date: 5/22/2001.
2. Duncan Ranch, Gabilan View. Salinas, California, Lot # 13. Water date: 4/18/2001. Evaluation date: 6/12/2001.
3. D' Arrigo Bros. Company. Salinas, California, Ranch # 5, lot 16A. Water date: 4/10/2001. Evaluation date: 6/18/2001.
4. D' Arrigo Bros. Company. Gonzales, California, Ranch # 7, lot 3. Water date: 8/7/2001. Evaluation date: 10/10/2001.

<sup>a</sup>Leaf area coefficient: Comparing leaf areas between the two varieties. This is calculated by multiplying the leaf width by the leaf length.

**Example #3**

'Big Star' is most similar to 'Two Star'; however, 'Big Star' is significantly more resistant to cupping (closure of internal leaves) than 'Two Star'.

Variable	Trial Location	No. Plants/ Rep	<sup>a</sup> No. 'Big Star' Cupped	No. 'Two Star' Cupped	<sup>b</sup> p [chi sq.]
Leaf Cupping	1	20	0	4	0.045
	2	20	0	4	0.045
	3	20	0	12	0.000
	4	20	0	9	0.004

Field Trial Locations:

1. D' Arrigo Bros. Company. Salinas, California, Ranch # 15, Lot # 29. Water date: 3/16/2001, Evaluation date: 5/22/01.
2. Duncan Ranch, Gabilan View. Salinas, California, Lot # 13. Water date: 4/18/2001. Evaluation date: 6/12/2001.
3. D' Arrigo Bros. Company. Salinas, California, Ranch # 5, lot 16A. Water date: 4/10/2001. Evaluation date: 6/18/2001.
4. D' Arrigo Bros. Company. Gonzales, California, Ranch # 7, lot 3. Water date: 8/7/2001. Evaluation date: 10/10/2001.

<sup>a</sup>Presence or absence of cupped plants was recorded as 1 and 0, respectively.

<sup>b</sup>Statistical analysis based on the probability of one-tailed Chi-squared distribution at 95% level.

**Example #4**

'Big Star' is most similar to 'Two Star'; however, 'Big Star' has more number of leaves per individual plant than 'Two Star'.

Variable	Trial Location	No. Plants/ Rep	t*-value	p[t*]	Avg. 'Big Star'	Avg. 'Two Star'	LSD Mean 95%	Sigma
No. Leaf per Plant	1	10	14.445	0.000	35.10	26.60	1.32	1.40
	2	10	8.534	0.000	30.50	25.50	1.31	1.39

Field Trial Locations:

1. D' Arrigo Bros. Company. Greenfield, California, Ranch # 10, lot 34. Water date: 8/1/2001. Evaluation date: 10/1/2001.
2. D' Arrigo Bros. Company. Gonzales, California, Ranch # 7, lot 3. Water date: 8/7/2001. Evaluation date: 10/10/2001.



**Statement of Distinctness Based on DNA Analysis**

"Big Star" is most similar to "Two Star"; however, when DNA analysis is performed, using PCR products of Genemed Synthesis, Inc. primer number 809, a DNA band of about 200 bp is absent in "Big Star" but present in "Two Star".

"Big Star" is most similar to "Two Star"; however, when DNA analysis is performed, using PCR products of Genemed Synthesis, Inc. primer number 824, a DNA band of about 200 bp is absent in "Big Star" but present in "Two Star".

"Big Star" is most similar to "Two Star"; however, when DNA analysis is performed, using PCR products of Genemed Synthesis, Inc. primer number 876, a DNA band of about 160 bp is absent in "Big Star" but present in "Two Star".

"Big Star" is most similar to "Two Star"; however, when DNA analysis is performed, using lettuce downy mildew linked SCAR primer SCH13, a DNA band of about 980 bp is absent in "Big Star" but present in "Two Star".

### DNA Analysis of Big Star and Two Star

**Introduction.** In general, current commercial lettuce types have low level of genetic diversity and that may create difficulties for lettuce improvement and simple identification. However, molecular markers [random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR) or microsatellites, and etc.] can be used for various genetic analysis such as genetic linkage map construction, genetic diversity studies, genetic trait identification, disease diagnostics tools and DNA fingerprinting (Doganlar et al. 1998, Kojima et al. 1998, Ratnaparkhe et al. 1998, Sicard et al. 1999). DNA markers have enabled characterization of genotypes independent of the influence of environmental growth conditions, physiological age of the plant and the type of tissue being analyzed. DNA markers are especially informative and superior to those revealed by traditional methods like morphological and protein based markers in determining genetic differences. Specific RAPD marker fragments have been successfully applied for identifying celery, blueberry, watermelon and rose cultivars (Yang and Quiros 1993, Aruna et al. 1995, Torres et al. 1993, Lee et al. 1996). In Chicory, *Cichorium intybus*, which is closely related to lettuce, RAPDs have been used for varietal identification and purity of F<sub>1</sub> hybrids (Bellamy et al. 1996). In lettuce, *Lactuca sativa* L., detailed genetic map has been constructed using various markers including RAPDs (Kesseli et al. 1994). Furthermore, RAPDs have been used in lettuce for accession variability and germplasm identification (Waycott and Fort 1994, Yamamoto et al. 1994) and to closely link DNA markers to various resistance genes, i.e. downy mildew, corky root, and mosaic virus, in lettuce (Paran et. al. 1991, Paran and Michelmore 1993, Robbins 1993, Michelmore 2000). Such linked amplified RAPD products are cloned and sequenced at both ends and turned into sequence characterized amplified regions (SCARs). SCAR primer pairs then amplify highly reproducible and robust single major DNA bands the same size as the RAPD fragment cloned (Paran and Michelmore 1993). In separate lettuce DNA extractions, RAPD banding pattern were assessed be very consistent in systematic blind tests and in across individuals within lines [Waycott and Fort 1994, Central Valley Seeds, Inc. (Unpublished Data)]. Therefore

identifying lettuce cultivars by the RAPD techniques is very well possible because each cultivar yields a reproducible DNA band pattern.

RAPD technique was used to distinguish varietal difference at the genomic DNA level and identify specific molecular marker bands between two leaf varieties, Big Star and Two Star.

**Plant Material and DNA Extraction.** Seeds of lettuce (*Lactuca sativa*) plants varieties Big Star (Central Valley Seeds, Inc., PVP pending application No. 200200013) seed lot No. GL-230-Y and Two Star (Orsetti Seed Company, Inc. Hollister, California, USA, PVP No. 9200270) seed lot No. 20-078-013 were germinated in the greenhouse. DNA was extracted from very young and actively growing leaves collected from pools of about 20 plants per variety using a modified CTAB method (Kesseli et. al. 1991). Genomic DNA was quantified using a DNA Fluorometer (Hoefer® DyNA Quant 200, Amersham Pharmacia Biotech, USA) and adjusted to 25 ng/μl in sterile TE buffer.

**DNA Analysis.** Genomic DNA was amplified with minor modifications as described by Waycott and Fort (1994) using decamer random oligonucleotide primers (Set #9) from Genemed Synthesis, Inc. (South San Francisco, CA, USA). RAPD DNA amplification conditions were performed on a Techne GeneMate® programmable thermocycler machine (Kaysville, UT, USA). DNA amplification was performed in 50 mM Tris-HCl (pH 9.1), 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 150 mg/ml BSA, 1.5 mM MgCl<sub>2</sub>, 2.5 mM dNTP, 4 mM primer, 25 ng of genomic DNA per 10 μl of reaction volume and 1 U of KlenTaq1® DNA Polymerase (Ab Peptides, Inc., St. Louis, MO, USA) for 1 cycle denaturation at 94° C for 2 min, followed by 40 cycles at 94° C for 1 min, 35° C for 1 min, and 72° C for 2 min, and a 5 min final extension at 72° C. The SCARs (sequence characterized amplified regions) linked to several important genetic traits in lettuce were amplified in conditions as described (Paran and Michelmore 1993). The amplified products were separated on 4% Latitude® HT precast agarose gels (BioWhittaker Molecular Applications, Rockland, Maine, USA) in 1X TBE buffer. Agarose gels were stained with ethidium bromide and scored for presence and absence of bands. To insure the reproducibility of the RAPD markers, genomic DNA from each variety was amplified separately at least four times at different dates.

**Results.** The amplification profiles were highly informative to establish unique DNA fingerprint for genomic differences between Big Star and Two Star lettuce varieties. The polymorphic primers were tested several times to examine the reproducibility of the amplification reactions. In the present work, DNA amplifications were obvious, reproducible and consistently resulted in the same RAPD profile. The summary of polymorphic bands data between Big Star and Two Star is presented in the Table 1. For example, primer 809 discriminated Big Star from Two Star by the presence and absence of a ~200 bp DNA fragment band in Two Star than Big Star, respectively (see Figures 1 and 2).

In review, the lettuce varieties Big Star and Two Star could be discriminated from each other based on their RAPD and SCARs profiles, which makes this technique useful for lettuce fingerprinting and illustrating genetic differences in analogous cultivars. From this initial study, our results do support our claim that Big Star and Two Star are different at the genomic level. Additional work may be needed to further apply this technique, when compared to other standard commercial lettuce cultivars, for lettuce variety protection, pedigree analysis and seed purity determination.

**Table 1. Polymorphic DNA markers and primer sequence used in the characterization of lettuce leaf cultivars Big Star and Two Star.**

Primer <sup>a</sup>	Primer Sequence (5'-3')	Polymorphic Band (~bp)	Big Star <sup>b</sup>	Two Star
809	GCC ATG TAC T	200	A	P
824	TCA AGG TCC T	200	A	P
876	TCC TGC CAG T	160	A	P
SCH13	F-GAC GCC ACA CAC ACA CTT TAA GTA R-GAC GCC ACA CCT ATA TCC TTT ACC	980	A	P

<sup>a</sup>“SC”, “F”, and “R” refer to SCARs, forward and reverse primers, respectively.

<sup>b</sup>“A” or “P” indicate the absence and presence of the polymorphic band, respectively.

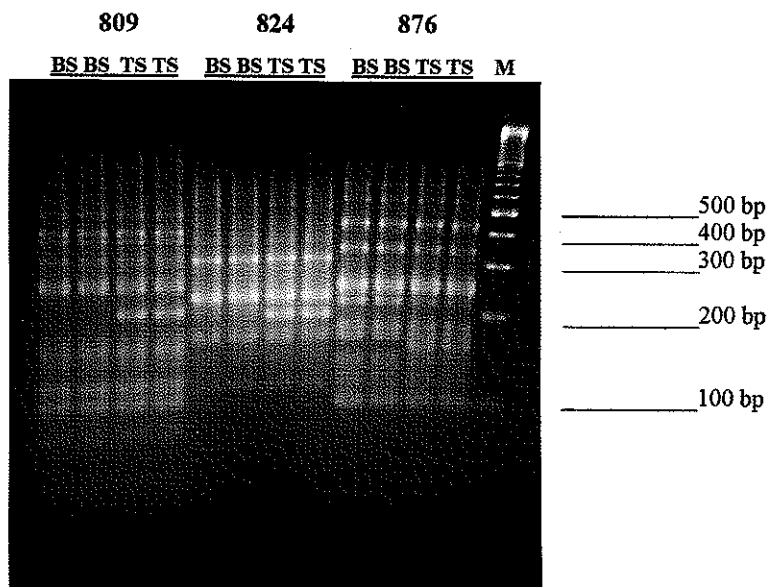


Figure 1. Variation of RAPD profiles between Big Star (BS) and Two Star (TS) lettuce varieties generated by Genemed primer Nos. 809, 824, and 876. Samples were run in duplicates. **M** is the size 100 bp DNA marker. Patterns highly reproducible.

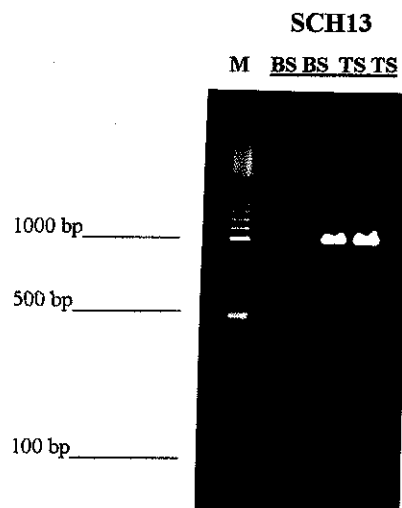


Figure 2. Variation of DNA profiles between Big Star (BS) and Two Star (TS) lettuce varieties generated by lettuce downy mildew link SCAR primer SCH13. Samples were run in duplicates. **M** is the size 100 bp DNA marker. Patterns highly reproducible.

## References

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**Exhibit E**  
**(Attachment)**

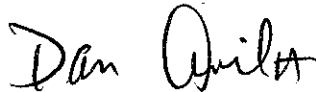
**Statement of the Basis of Applicant's Ownership**

October 11, 2001

Tony M. Avila, Research and Development Director/ Plant Breeder and Adolfo Mederos developed the lettuce variety 'Big Star' that has been entered in this Plant Variety Protection application for Central Valley Seeds, Inc.

Tony M. Avila, Dan M. Avila, Joe M. Avila and John M. Avila equal owners of Central Valley Seeds, Inc. own 'Big Star'. The ownership name of the PVP certificate shall be under the name of **CENTRAL VALLEY SEEDS, INC.**

Sincerely,



Dan Avila  
Central Valley Seeds, Inc.

U.S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL MARKETING SERVICE

EXHIBIT E  
STATEMENT OF THE BASIS OF OWNERSHIP

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF APPLICANT(S) RAO 7/28/05 <del>TONY AVILA, DAN AVILA, JOE AVILA,</del> <del>JOHN AVILA</del> CENTRAL VALLEY SEEDS, INC.		2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER G.L. 230-1-M-M	3. VARIETY NAME BIG STAR
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code, and Country) 485 VICTOR WAY, SUITE 10 SALINAS, CA. 93907		5. TELEPHONE (include area code) 831 757-0939	6. FAX (include area code) 831 757-6829
		7. PVPO NUMBER 200200013	

8. Does the applicant own all rights to the variety? Mark an "X" in appropriate block. If no, please explain.

☒ YES ☐ NO

9. Is the applicant (individual or company) a U.S. national or U.S. based company?  
If no, give name of country

☒ YES ☐ NO

10. Is the applicant the original owner? ☒ YES ☐ NO If no, please answer the following:

a. If original rights to variety were owned by individual(s), is (are) the original owner(s) a U.S. national(s)?

☐ YES ☐ NO If no, give name of country

b. If original rights to variety were owned by a company, is the original owner(s) a U.S. based company?

☐ YES ☐ NO If no, give name of country

11. Additional explanation on ownership (If needed, use reverse for extra space):

SEE ATTACHED SHEET OF EXHIBIT E  
STATEMENT OF THE BASIS OF APPLICANT'S OWNERSHIP

## PLEASE NOTE:

Plant variety protection can be afforded only to owners (not licensees) who meet one of the following criteria:

1. If the rights to the variety are owned by the original breeder, that person must be a U.S. national, national of a UPOV member country, or national of a country which affords similar protection to nationals of the U.S. for the same genus and species.
2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by nationals of a country which affords similar protection to nationals of the U.S. for the same genus and species.
3. If the applicant is an owner who is not the original owner, both the original owner and the applicant must meet one of the above criteria.

The original breeder/owner may be the individual or company who directed final breeding. See Section 41(a)(2) of the Plant Variety Protection Act for definition.

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 10 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in its programs on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, and marital or familial status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (braille, large print, audiotape, etc.) should contact the USDA Office of Communications at (202) 720-5881 (voice) or (202) 720-7808 (TDD). To file a complaint, write the Secretary of Agriculture, U.S. Department of Agriculture, Washington, D.C. 20250, or call 1-800-245-6340 (voice) or (202) 720-1127 (TDD). USDA is an equal employment opportunity employer.